UNIVERSITI TEKNOLOGI MARA

ANTIPROLIFERATIVE EFFECT OF *CASSIA AURICULATA* (CAESALPINIACEAE) FLOWERS

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Thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science

Faculty of Pharmacy

August 2014

AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for attainment of any degree or qualification.

I hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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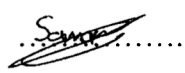
Signature of Student	:	
Date	:	August 2014

ABSTRACT

Cassia auriculata is a common South Asian medicinal plant which is widely used in traditional medicine. The plant has been used in folk medicine to treat various disease conditions and the pharmacological activity of different parts of the plant has been well documented as anti-diabetic, anti-oxidant and anti-hyperlipidemic. This study was aimed at investigating anti-proliferative activity of *C. auriculata* flowers. Cellular viability was measured by (MTS) assay. Effects on apoptotic (programed cell death) machinery were examined using annexin-V and propidium iodide staining using flow cytometry. Molecular mechanisms were analyzed by real-time polymerase chain reaction (RT-PCR). Effects on cell cycle were determined by cell cycle analysis using flow cytometry. Crude extract of *C. auriculata* flowers was prepared by extraction with a mixture of methanol and dichloromethane. The results showed that crude extract exhibited specific anti-proliferative effect against liver cancer (HepG2) cell line with IC_{50} value of 63±2.2 µg/ml as compared to effects on mammary cancer (MCF-7) and colon cancer (HCT116) cell lines with IC_{50} values of 125±2.6 and 199±5.8 µg/ml, respectively. Crude extract was selective to liver cancer cells as it showed minimal cytotoxic effect against normal embryonic liver (WRL-68) cell line with IC_{50} of 251±4.1 µg/ml. Partitioning and fractionation of crude extract yielded six fractions of which, fraction1 elicited most potent anti-proliferative effect in HepG2 cells with an IC_{50} of 12.5± 2.3 µg/ml. Mechanism of cell death was via apoptosis which was significantly induced in a dose-dependent manner in HepG2 cells treated with crude extract or with fraction1 at three different concentrations which corresponded to their inhibitory concentrations in HepG2 cells (IC_{20}, IC_{50} and IC_{70}). By comparison, fraction1 was more potent that crude extract at induction of apoptosis. Induction of apoptosis by both crude extract and fraction1 occurred via up-regulation in expression levels of p53, a tumor suppressor gene; Bax, a pro apoptotic gene; caspase-3, a major apoptotic gene and simultaneous down-regulation of Bcl-2, an anti-apoptotic gene. Cell cycle analysis showed crude extract and fraction1 elicited arrest of cells at G_2/M phase. Treatment of HepG2 with F1 caused marked accumulation of cells at G_2/M phase and a corresponding decrease in cells at G_0/G_1 and S phases. These results show that *C. auriculata* flower extract and its purified fraction exerted anti-proliferative effect in HepG2 cells via induction of apoptosis and arrest of the cell cycle at G_2/M phase. There is potential to further develop *C. auriculata* flowers for adjuvant management of hepatocellular carcinoma. More work is required to examine the phytochemistry of *C. auriculata* in relation to its anti-proliferative effect in liver cancer cells.

ACKNOWLEDGEMENTS

First and foremost, I would like to pay my gratitude to Allah S.W.T for giving the strength and His blessing in complete my Master's thesis.

I would like to express my deepest gratitude and appreciation to my supervisor Professor. Dr. Aishah Adam, who imparted her expertise, knowledge and guidance, and give me the opportunity to do this research and her unconditional support at all levels throughout the project as whole.

I wish to express my sincere thanks to my co-supervisor Dr.Wan Iryani for her valuable advice, continuous support and kind encouragement throughout my study.

My sincere and special thanks to Dr. Ghanya Al-Naqeb for her invaluable help, support, understanding and technical assistance through this project.

My warmest thanks to my lab mates for their invaluable help and kind in the laboratory. Specially, Manizheh Khalilpour, Nuraini Aziz, Khairunnisa Razali and Carol Ntimi for their unfailing encouragement and never ending friendship and enjoyable company. I would like to extend my thanks to Mrs Cini Mathew for offering the plant used in this study and to my lab mates Aidil Azahar, Saad Zamani, Farhana Abdualrahman and Wesam Radhi for their help and assisting in my lab work.

Iam thankful to my wonderful friends for sharing many happy moments and their moral support during difficult times. Specially, Hazar Saleh, Najla Layas and Zahra Bashir.

Last but not least, this thesis dedicated to my family, without whom none of this would have been possible. Iam deeply grateful to my dear parents, dear brother and dear sisters for always supporting me unconditionally and love throughout my life.

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